Journal of Oriental Medicine Industry
2009, Vol.1, No.1, pp.75~80

Antifungal Activity and Phytochemical Study of Selected Medicinal Plants in East Kalimantan

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ABSTRACT

Plants having long history in traditional health care application represent an understandable choice for scientifically investigation. It is fascinating to determine whether the traditional uses are supported by actual biological effects or merely based on folklore. In the present study, 15 plants currently used as folk medicine in East Kalimantan, Indonesia were investigated their antifungal activity against a dermatophyte, *Trichophyton mentagrophytes*. Leaves of the selected plants were extracted with methanol in room temperature. The methanolic extracts obtained weresubjected to antifungal assays using *Trichophyton mentagrophytes* as a test fungus. In the agar diffusion antifungal assays, *Zingiber purpureum*, *Eleutherine americana*, *Leucaena glauca*, *Eugenia polyantha*, and *Hibiscus tiliaceus* showed more activity than others by exhibiting 35-61% inihibitory activity in comparison to that of a commercial antifungal agent, miconazole. Further phytochemical study of the plants potential to be used as antidermatophyte agents showed that *Zingiber purpureum* contains alkaloid, steroid, flavonoid and carbohydrate;

Key words: antifungal activity, bioassays, dermatophyte, East Kalimantan, phytochemical study, plant extracts.

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Eleutherine americana contains alkaloid, steroid, flavonoid and carbohydrate, *Leucaena glauca* contains alkaloid, steroids and carbohydrate, *Eugenia polyantha* contains alkaloid, triterpenoid, saponin, flavonoid and carbohydrate; and *Hibiscus tiliaceus*contains alkaloid, triterpenoid, flavonoid and carbohydrate.

1. Introduction

Tropical forest of Indonesia covers 110 million hectares occupied by about 80% of world medicinal plants. It is estimated that in the Indonesian tropical forest, more than 28.000 plant species exist and 1.000 species of them have been known and already used for medicinal purposes[1)]. In general, investigation into the efficacy of Indonesian medicinal plants is still limited on folklore information of the utilization of the plants by local people.

East Kalimantan is an island in Indonesia having a huge tropical forest along with Sumatera and Papua. The plant biodiversity exist in the island promises high potential of biological activities, including for the searching of antifungal agents. Regarding antifungal properties, several plants have been investigated for their ability to inhibit fungal growth, e.g.: *Terminalia catappa*, *Phyllanthus acuminatus*, *Ipomoea* spp. *Tylophora asthmatica*, *Hyptis brevipes*[2)], and *Zingiber officinale* Roscoe. [3)].

In the course of our search into natural antifungal agents, the present paper reports the potential of some medicinal plants in East Kalimantan, Indonesia to inhibit the growing of *Tricophyton mentagrophytes*, a fungal belongs to dermatophyte and the phytochemical analysis of the plants.

2. Materials and methods

2.1. Plants samples

The plants were collected from East Kalimantan on the basis of their ethnobotanical information (Table 1). The plant were identified by a taxonomist of our university. The voucher specimens were deposited in our laboratory.

2.2. Fungal culture

Trichophyton mentagrophytes was used as a test fungus. The isolates were subcultured onto potato dextrose agar (PDA) plates at 28° C. Stock inoculum suspensions of each isolate were prepared for each experiment from 7 to 14 day old cultures grown

on PDA. The fungal colonies were covered with ca. 5 ml of distilled water, and suspensionswere made by gently probing the surface with the tip of a Pasteur pipette. The resulting mixture of conidia and hyphal fragments was filtered and transferred to a sterile tube. The densities of these suspensions were adjusted with a spectrophotometer at a transmittance of 75%.

2.3. Extraction

Plant powders (ca. 100 g) were extracted with metanol for 48 hours at room temperature. The extraction was performed three times. Extract solution was filtered with a Whatman no 2 (Whatman International Ltd., England). The solvent was removed with a rotary evaporator at 40°C under reduced pressure. The extract was kept in a vacuum oven to dryness to give solid extracts. Each respective soluble fraction was subjected to an antidermatophyte activity assay against *T. mentagrophytes*.

2.4. Antidermatophyte assay

Antidermatophyte assay was conducted with agar diffusion on a paper disc. Twenty-milliliter aliquots of sterile molten PDA were transferred to Petri dishes and allowed to solidify. The PDA plates were inoculated with 100 mL of inocula suspension spread

Table 1. Plant samples

No	Local Name	Vernicular Name	Family	Part Used
1.	Asam Jawa	*Tamarindus indica* Linn.	Leguminosae	Leaves
2.	Bangle	*Zingiber purpureum* Roxb.	Zingiberaceae	Rhizome
3.	Bawang Tiwai	*Eleutherine americana* L. Merr.	Iridaceae	Leaves
4.	Beringin	*Ficus benjamina* Linn.	Moraceae	Leaves
5.	Jambu Biji	*Psidium guajava* Linn.	Myrtaceae	Leaves
6.	Ketepeng Cina	*Cassia alata* L.	Leguminosae	Leaves
7.	Kumis Kucing	*Orthosiphon stamineus* Benth.	Labiatae	Leaves
8.	Kunyit Hitam	*Curcuma aeruginosa* Roxb.	Zingiberaceae	Rhizome
9.	Kupu-kupu	*Bauhinia tomentosa* L.	Leguminosae	Leaves
10.	Lamtoro	*Leucaena glauca* Linn.	Fabaceae	Leaves
11.	Lidah buaya	*Aloe vera Linn*	Liliaceae	Leaves
12.	Pecut Kuda	*Stachytarpheta jamaicensis* (L) Vahl.	Verbenaceae	Leaves
13.	Salam	*Eugenia polyantha* Wight.	Myrtaceae	Leaves
14.	Tanjung	*Mimusops elengi* L.	Sapotaceae	Leaves
15.	Waru	*Hibiscus tiliaceus* L.	Malvaceae	Wood

uniformly on the surface of the plates. A sterile paper disc (6 mm i.d) containing 10 mL of extract (equals to 2 mg extract) was applied to the surface of each inoculated plate. The plates were incubated in the dark at 25°C for 48 hours. Zones of inhibition around the discs were measured in mm. The control plate contained acetone and medium only. Miconazole (10 mg/disc) was used as a positive control. Incubation was conducted for 5 days.

2.5. Phytochemical analysis

One gramof the methanol extracts of the plants was dissolved in the methanol to obtain a stock concent-ration 1%. The extracts obtained were subjected to a phytochemical analysis following the method as described[4,5].

3. Results and discussion

The extract result and phytoche-mical analysis of some Indonesian medicinal plants was presented in Table 2.

Of fifteen plant extract tested, thirteen plants contain alkaloids, six plants contain triterpenoids, nine plants contain steroids, four plants contain saponins, twelve plants contain flavonoid and whole plants contain carbohydrates. The results showed that the plants contain a broad range of phytochemicals including alkaloids, triterpenoids, steroid, saponins flavonoid and carbohydrates. The results of the phytochemical analysis will support the utilization of the respective plants. For example, flavonoids have been reported to have antimicrobial properties[6], saponins showed antimicrobial activity, while alkaloids have displayed physiological effect particularly in the nervous system[7].

Antidermatophyte assay of some Indonesian medicinal plants was conducted against *T. mentagrophytes* and the result was presented in Table 3. The result showed that 5 plants possess potential activity against *T. mentagrophytes*, i.e.: Bangle (*Zingiber pupureum* Roxb.), Bawang Tiwai (*Eleutherine Americana* L. Merr.), Lamtoro (*Leucaena glauca* Linn.), Merbau (*Intsia palembanica* Miq.), Salam (*Eugenia polyantha* Wight.), Sirih (*Piper betle*, Linn.) dan Waru (*Hibiscus tiliaceus* L) with the activity ranged from 35% (*L. glauca*) to 61% (*E. polyantha*).

Table 2. Phytochemical analysis of some Indonesian medicinal plants.

			Phytochemicals					
			Al	**Tr**	**St**	**Sa**	**Fl**	Ca
1.	*T. indica*	15.2	++	++	−	−	+	+++
2.	*Z. purpureum*	2.8	++	−	++	−	++	+++
3.	*E. americana*	6.6	+	−	+++	−	+	+
4.	*F. benjamina*	4.6	++	−	−	+	+	++
5.	*P. guajava*	9.5	+	+++	−	−	+++	+++
6.	*C. alata*	7.3	++	−	+++	+	+	+++
7.	*O. stamineus*	14.5	++	−	+++	−	+	++
8.	*C. aeruginosa*	12.3	++	−	−	−	−	+
9.	*B. tomentosa*	2.6	+	+++	−	−	++	+++
10.	*L. glauca*	29.6	+	−	+++	−	−	+++
11.	*A. vera*	7.9	−	−	−	−	−	++
12.	*S. jamaicensis*	7.4	−	−	−	+++	+	+
13.	*E. polyantha*	31.6	++	−	++	−	−	+
14.	*M. elengi*	6.0	+	+++	−	+	+	+
15.	*H. tiliaceus*	15.8	+	++	−	−	+	+++

*Based on a dry weight basis, al=alkaloid, tr=triterpenoid, st=steroid, sa=saponin, fl=flavonoid, Ca=carbohydrate.

Table 3. Antifungal activity of some Indonesian medicinal plants against *Tricophyton mentagrophytes*.

No	Plant samples	**Part used**	Antifungal activity (%) vs Miconazole*
1.	*T. indica*	Leaves	0
2.	*Z. purpureum*	Rhizome	53 ± 0,3
3.	*E. americana*	Leaves	50 ± 0,2
4.	*F. benjamina*	Leaves	0
5.	*P. guajava*	Leaves	0
6.	*C. alata*	Leaves	0
7.	*O. stamineus*	Leaves	0
8.	*C. aeruginosa*	Rhizome	0
9.	*B. tomentosa*	Leaves	0
10.	*L. glauca*	Leaves	35 ± 0,2
11.	*A. vera*	Leaves	7
12.	*S. jamaicensis*	Leaves	0
13.	*E. polyantha*	Leaves	61 ± 0,4
14.	*M. elengi*	Leaves	0
15.	*H. tiliaceus*	Wood	47 ± 0,1
16.	Miconazole	−	100

* Average value of triplicates ± standard error. Extract amount was 2 mg /paper disc. Miconazole was used as a positive control.

The results of the present research indicated an opportunity to use the Indonesian medicinal plants as natural antifungal agents. Isolation and identification of the active compounds from the potential plants are in progress in our laboratory.

Acknowledgement

A support from PT. Kaltim Methanol Industri for providing some chemicals is gratefully acknowledged.

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